SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. GENERAL INFORMATION

Generic Name of the Device

Cervical cytology slide preparation device

Trade Name of the Device

The AutoCyte PREP™ System (CytoRich® Preparation Process)

Applicant's Name and Address

AutoCyte, Inc.
780 Plantation Drive
Burlington, NC 27215

Premarket Approval Application (PMA) Number **P970018**

Date of Panel Recommendation

Pursuant to section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Hematology and Pathology Devices Advisory Panel meeting because the information in the PMA substantially duplicated information previously reviewed by this panel.

Date of Notice of Approval to the Applicant

June 17, 1999

II. INDICATIONS FOR USE

Intended Use

The AutoCyte PREP™ System is a liquid-based thin-layer cell preparation process. The AutoCyte PREP™ System produces slides that are intended as replacements for

8

conventional gynecologic Pap smears. The AutoCyte PREP™ System slides are intended for use in the screening and detection of cervical cancer, pre-cancerous lesions, atypical cells and all other cytologic categories as defined by The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses.[1]

PATIENT POPULATION

The intended population consists of all women who are screened for cervical neoplasia or its precursor lesions.

BACKGROUND

The Cervical Pap smear was developed by Dr. George Papanicolaou[2] over 50 years ago as a means of recognizing cellular morphological change that is consistent with cancer and pre-cancerous conditions. The broad use of the Pap smear in routine cervical cancer screening is credited with a reduction in cervical cancer death rates of 70 percent.[3,4]

III. DEVICE DESCRIPTION

A. Device components

The AutoCyte PREP™ System device components include:

CytoRich® Preservative Fluid Collection Vial

Cervex-Brush® (Rovers Diagnostic Devices, Oss, The Netherlands)

CytoRich® Preservative Fluid

CytoRich® Density Reagent

CytoRich® Slide Coat

AutoCyte PREP™ CyRinges

AutoCyte PREP™ Settling Chambers

Cytology Stain Kit

AutoCyte PREP™ Glass Slides

Centrifuge Tubes

Slide and Tube Racks

Disposable Transfer and Aspirator Tips

B. Device Operations

The AutoCyte PREP™ System is a device with components for converting a liquid suspension of cervical cells into a consistently stained thin-layer of cells and cell clusters. The process includes cell preservation, randomization and enrichment of diagnostic clinical material, automated pipetting, sedimentation and discrete staining, to create a thin-layer cellular preparation. The AutoCyte PREP™ is a robotic pipetter which automatically performs the mixing, transfer, sedimentation, and staining portions of the process.

The AutoCyte PREP™ System is semi-automated. A patient's gynecologic specimen is collected by qualified medical personnel using a broom-type sampling device. The end of the device is removed and immediately immersed into AutoCyte Preservative Fluid. The vial is capped, labeled and sent with appropriate paperwork to the laboratory for processing.

At the laboratory, the collected cells are disaggregated by the shearing forces of vortexing and syringing yielding a mixed liquid cell suspension. The cell suspension is then layered onto a density reagent (CytoRich® Density Reagent) in a centrifuge tube. Centrifugal sedimentation of the cell suspension through the density reagent partially removes debris and reduces the number of inflammatory cells from the specimen. The density reagent is decanted. The centrifuge buckets containing the centrifuge tubes with cell pellets are placed onto the AutoCyte PREP™ System. From this point, the robotic processor takes over the process, performing the liquid transfers, cell sedimentation, and discrete staining steps automatically.

Using its program controlled pipette and delivery system, the AutoCyte PREP™ System mixes and transfers samples of the cell pellets to small plastic columns (AutoCyte PREP™ Settling Chambers) mounted on cationically coated microscope slides. The cells are then allowed to settle by gravity for a minimum of 10 minutes. Following this step, AutoCyte PREP™System automatically stains the cells discretely in their individual chambers using a modified Papanicolaou staining method. The chambers are then removed and the slides are coverslipped in preparation for screening.

IV. CONTRAINDICATIONS, WARNINGS AND PRECAUTIONS

There are no known contraindications. See labeling for warnings and precautions

V. ALTERNATE PRACTICES AND PROCEDURES

The Papanicolaou Smear

The conventional Papanicolaou smear is the primary procedure for screening the population for cervical neoplasm or its precursor lesions. It consists of scraping cells from the patient's cervix and manually spreading them onto a glass slide for examination.

There is an approved PMA for one other thin-layer liquid based preparation device.

VI. MARKETING HISTORY

Since January 1995, this device has been marketed in Canada, Australia, Germany, France, Switzerland Philippines, Denmark, Portugal, Italy, Belgium, Japan, Holland, Hong Kong, and the UK for non-gynecological and gynecological use. The AutoCyte PREP™ System has not been removed from the market for any reason related to the safety and effectiveness of the device.

VII. POTENTIAL ADVERSE EFFECTS ON HEALTH

Specimen preparation errors may result in false negative or false positive diagnoses. A false negative diagnosis results in a delayed diagnosis and treatment for the patient. A false positive diagnosis may result when a slide is interpreted as containing abnormalities when no disease is present. As a result the patient may have an unnecessary colposcopic exam (which is a non-invasive procedure) or may be referred for a biopsy (which is an invasive procedure).

VIII. SUMMARY OF STUDIES

Non-Clinical Laboratory Studies

The non-clinical laboratory studies were designed to assess the (1) Performance Characteristics, (2) Reagent and Sample Stability, and (3) Process Qualification of the AutoCyte PREP™ System. These studies evaluated the variation of the system within a

11

single assay, among multiple assays, among multiple sites, and among replicate slides prepared from a single specimen or pooled specimens.

(1) Performance

Studies to evaluate performance characteristics investigated variability (reproducibility) within a single processing run, among multiple runs performed at a single site, among runs performed at multiple sites, and among multiple slides made from a single sample. In three variability studies, replicate aliquots from pools of cytologic material with a range of diagnostic characteristics were processed. In three other studies, slides prepared from individual specimens were used to evaluate sensitivity and reproducibility. Even though pooled material was used, all specimens were processed according to the standard AutoCyte PREP™ System procedure. The resulting slides were evaluated using one or more of the following measures: specimen adequacy (as determined by the presence of an endocervical component); diagnostic agreement; numbers of epithelial and inflammatory cells; numbers of abnormal cells on slides prepared from pools containing greater proportions of abnormal specimens; and quality of the preparation in five areas: Particulates and Debris, Overall Color and Hue, Nuclear Detail, Cytoplasmic Differentiation, and Cluster Architecture.

An endocervical component was seen on all slides prepared in the site-to-site, run-to-run, and within run studies. Diagnostic agreement was good, with a mean agreement within one category of 92% - 100%. Numbers of epithelial, inflammatory, and abnormal cells were relatively consistent within each pool among the runs. Preparation consistency was good in the categories Overall Color and Hue, Nuclear Detail, Cytoplasmic Differentiation, and Cluster Architecture. More variation was seen in the Particulates and Debris category; however, the variation was determined to be acceptable.

The slide variability study showed acceptable variation in the numbers of epithelial, inflammatory, and endocervical cells among the four slides prepared from each patient. The %CV was less than 25% in most (17 of 26, or 65%) patients for epithelial and inflammatory cells. Endocervical cells were present on all four of the slides made from 25 of the 26 patient specimens, for a 96% agreement. Eleven of 12 patients with ASCUS/ AGUS cells (92%) had the atypical cells on each of the four slides; the 12th patient (8%) had atypical cells on three of the four slides. When LSIL, HSIL, or cancer cells were seen on one of a patient's slides, these abnormal cell types were present on all four of the slides from that patient. The study showed that variability among the four slides prepared from a single specimen had no impact on diagnostic interpretation.

The sensitivity study showed that diagnoses of thin-layers made from abnormal specimens diluted in a pool of normal cells was not significantly affected. Diagnostic agreement was 100% at all dilutions for 8 of the 10 patients. When all dilutions for all patient specimens are considered, 100% agreement was found in 50 of the 60 slide sets (83%). All slides in all sets were diagnosed as abnormal (LSIL, HSIL, or cancer).

The final non-clinical performance study demonstrated the appropriateness of the amount and randomization of material presented on the thin-layer preparation. These data indicate that clinically relevant cervical cells, endocervical components, and microorganisms are represented in numbers sufficient to be diagnosed even when only half of the CytoRich® thin-layer preparation is reviewed.

(2) Reagent and Sample Stability

Because CytoRich® Preservative Fluid, CytoRich® Slide Coat, and CytoRich® Density Reagent are involved in different aspects of the process, the variability of each was assessed independently. A "split brush" collection method was used in which the sampling brush was cut in half. One portion was placed in the test vial of CytoRich® Preservative Fluid and the other in the control vial of CytoRich® Preservative Fluid. Pooled specimens were used to evaluate the density reagent and slide coat reagent.

The slides prepared in each of the stability/lot-to-lot variability studies were evaluated using one or more of the following measures: specimen adequacy (as determined by the presence of an endocervical component), diagnostic agreement, numbers of epithelial, inflammatory, and abnormal cells, and quality of the preparation in five areas: Particulates and Debris, Overall Color and Hue, Nuclear Detail, Cytoplasmic Differentiation, and Cluster Architecture.

In addition, the stability studies included testing for anti-microbial preservative effectiveness (APE) and for microbial load. To assess APE, aliquots of Preservative Fluid and of slide coat were challenged with five organisms (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Aspergillus niger*). To assess microbial load, samples of slide coat and of density reagent were cultured in duplicate for the presence of yeast, mold, and aerobic bacteria. The number of colonies on each plate was counted to yield the number of CFUs per ml of reagent.

The results for these studies are as follows:

- Shipping Cycle Stability

The three kits, each containing one lot of preservative fluid, density reagent, and slide coat, were subjected to a series of temperature fluctuations intended to simulate "worst case" shipping conditions. The test vials were processed using the test density reagent and slide coat and the control preservative fluid vials were processed using control reagents. The slide pairs from each patient were compared for specimen adequacy, diagnostic agreement, numbers of epithelial and inflammatory cells per slide and quality of preparation based on debris and particulates, overall color and hue, nuclear detail, cytoplasmic differentiation and cluster architecture. The results of the study showed diagnostic agreement within one Bethesda System category in 79% - 100% of slides; presence of an endocervical component on all slides; numbers of epithelial and inflammatory cells comparable between test and control slides; and preparation quality was comparable with 82% - 100% of test slides as good as the control slides.

- Stability and Lot-to-Lot Variability Preservative Fluid

The results of the study comparing aged vials of preservative fluid with a fresh control vial showed diagnostic agreement within one category of 86% - 93%. Preparation quality was comparable, with 90% - 96% of test slide ratings for all categories combined being as good as or better than the control slide ratings. The preservative fluid was shown to be an effective anti-microbial preservative and slides made from samples collected in each of the three lots of CytoRich® Preservative Fluid were comparable to the control, showing that the reagent is stable through its labeled dating period of one year. Differences among slides prepared from specimens collected in the three test lots of preservative fluid did not impact clinical interpretation.

- CytoRich® Slide Coat Stability and Lot-to-Lot Variability

This study tested replicate samples from two different pools of normal and abnormal cytologic specimens processed with three different aged test lots and a fresh control lot of slide coat. Study results show 100% agreement within one diagnostic agreement; presence of an endocervical component on all slides; comparable numbers of epithelial and inflammatory cells on test and control slides; and similar number of abnormal cells on all slides. The slide coat was found to be bacterial static as colonies of *A. niger* were seen but the concentrations remained at or below the initial concentrations. Slides made using each of the test lots of slide coat were comparable to the control slide.

- CytoRich® Density Reagent and Lot-to-Lot Variability

Slides made from the aged reagents and evaluated for the usual characteristics study results show 100% agreement within one diagnostic agreement; presence of an endocervical component on all slides; comparable numbers of epithelial and inflammatory cells on test and control slides; and similar number of abnormal cells on all slides. The density reagent was found to be bactericidal showing only one colony of bacteria on one of two plates tested. Slides made using each of the test lots of density reagent were comparable to the control slide.

- Stability of Cytologic Materials Stored in CytoRich® Preservative Fluid

The results from the comparison of slides prepared within a short time of specimen collection and slides prepared from the same materials after longer term storage indicate that diagnostic agreement and overall preparation quality were good. All 22 slide pairs in the short-term storage phase and 30 of the 31 slides pairs in the long-term storage phase agreed within one diagnostic category and 90%-95% of test slide ratings were as good as those of the control. Storage in CytoRich® Preservative Fluid for up to 6 months did not appear to impact the diagnostic utility of cytologic materials.

(3) Process Qualification

Qualification studies were performed to evaluate the relationship of variables in each of the three major preparation steps: sample randomization; enrichment of cell suspension; and thin-layer formation. The seven studies focused on vortexing, enrichment centrifugation, gravity sedimentation, slide coat formulation, particulate debris removal, and enrichment studies. Numbers of epithelial, inflammatory, and abnormal cells were evaluated in the sample randomization and thin-layer formation studies while numbers and size of particles in the various centrifugation fractions were evaluated in the enrichment of cell suspension studies. These studies evaluated the variables associated with each step in order to determine the optimum parameters and the tolerances to variation.

- Vortexing

The vortexing step is used to randomize and mix the sample, disassociate mucus and break up large cell sheets. The vortex step was evaluated for time and vortex speed. Vortex times of 5 to 30 seconds with vortex speeds of 1500 and 3000 rpm yielded preparations with acceptable cellularity and numbers of diagnostic cells. This study showed that there was an increase in the number of epithelial and inflammatory cells per slide with increased vortex times and speed. The numbers of abnormal cells per slide were relatively consistent for all time and speed combinations tested. The standard CytoRich® Preparation Process, used in the clinical trials, requires a 15 second vortex time at a vortex speed of 3000 rpm and is supported by these data.

- Enrichment Centrifugation

Enrichment centrifugation is used to remove excess debris and inflammatory cells. Studies were designed to evaluate time and centrifugal force. It was found that the majority of diagnostic material is present at or below the interface of the preservative fluid and the density reagent. Particulate debris and inflammatory cells are present in the supernatant fluid above the interface.

Particulate Debris Removal

This study was designed to evaluate removal of extraneous debris during enrichment centrifugation. The removal of small particles (aggregated protein, disrupted membranes, microbial and red cell artifact, etc.) by density reagent centrifugation is an important step in the preparation of CytoRich® thin-layers. The evaluation of particulate debris removed in the centrifugation fractions was by particle size. A Coulter Multisizer Counter (Coulter Diagnostics, Hialeah, Florida) was used to quantitate debris particles in the various enrichment fractions. The study showed that small particles 3.3μ - 6.6μ are equivalent to non-clinical debris; medium particles 6.1μ - 11.0μ represent inflammatory cells and large debris; and large particles 11.1μ - 60.0μ represent epithelial, endocervical, and other clinically significant cells. The results show that approximately 80% of debris-sized particulates in the 3.3 μ - 6.0μ range were removed in the supernatant and interface enrichment fractions.

Centrifugal Force

The results showed that the enrichment process was effective for g force combinations ranging from 150 to 600 x g for 1 to 5 minutes. The standard AutoCyte PREP™ System, used in the clinical trials, requires enrichment centrifugation for 2 minutes at 200 x g and is supported by these data.

- Gravity Sedimentation

Gravity sedimentation is used to layer cervical cells onto the CytoRich® thin-layer preparations. These studies were used to evaluate the variables of settling time, volume of fluid in the settling chamber, and cell concentration on the diagnostic quality of the CytoRich® thin-layer. Replicate aliquots of a pool of normal cervical samples were processed using a variety of settling times from <1 minute to 20 minutes. Epithelial and inflammatory cells were counted for all slides with the conclusion that the relative proportions of these cells are not affected by varying the settling times.

The second phase of the study processed aliquots of normal cervical sample pool substituting a different volume of deionized water for the standard 600 µl. The relative proportions of epithelial and inflammatory cells were not effected by varying the volume rinsed into the settling chamber after cell transfer. Settling fluid volumes tested varied from 200 to 1000 µl.

The third phase of this study evaluated the individual and combined influence of the three variables; settling time, fluid volume, and cell concentration with the conclusion that settling and fluid volume had a slight influence on the numbers of cells on the slide. The concentration of the cell suspension did influence the numbers of epithelial and inflammatory cells, but had little influence on the numbers of abnormal cells.

Epithelial cell concentrations in the settling fluid varied from 25,000 to 300,000. In addition, three abnormal cell pools were also evaluated. The variables of the parameters studied had very little influence on the cellularity and diagnostic acceptability of CytoRich® thin-layer preparations. This lack of influence allows the AutoCyte PREP™ System a high degree of latitude in the settling times, liquid settling volumes and routine cell concentrations used. The standard AutoCyte PREP™ System used in the clinical trials requires the final volume in the settling chamber of 600 µl, a settling time of 10 minutes and is supported by these data.

- Slide Coat Formulation

CytoRich® Slide Coat is a cationic poly amino acid peptide that is used to bind cells to glass microscope slides. These studies were designed to evaluate three parameters associated with the use of the slide coat. The variables include cell concentration, slide coat pH and concentration. Epithelial cell concentration studied, ranged from 25,000 to 200,000; slide coat pH ranged from 5.0 to 10.0 and slide coat concentration ranged from 0.01% to 0.1%.

The results showed that none of the three variables studied had a significant influence on the cellularity and diagnostic acceptability of the CytoRich® thin-layer preparations concluding that acceptable numbers of epithelial cells (5,650-121,080) were present at pH values from 7.0 to 10.0 and at slide coat concentrations from 0.045% to 0.07%. Increased cell concentration had no significant effect on the epithelial cell numbers but did influence the number of inflammatory cells. The standard AutoCyte PREP™ System used in the clinical trials requires a CytoRich® Slide Coat formulation with pH 9.0 and a concentration of 0.06% and is supported by these data.

- Enrichment Studies

In order to optimize the number of diagnostically important cells on CytoRich® thin-layers, excess white cells, blood artifact, bacteria and debris are removed by density reagent enrichment centrifugation. The majority of diagnostic material is present at or below the interface of the preservative fluid and the density reagent. Particulate debris and inflammatory cells are present in the supernatant fluid above the interface. After centrifugation, this supernatant and interface and a small portion of the density reagent are removed and discarded. During a second centrifugation, the cells form a pellet at the bottom of the tube and the supernatant fluid is removed. Specimens from 19 patients with previously diagnosed HSIL or cervical cancer were used to study the four centrifugation fractions: the first supernatant; the first interface; the second supernatant (decanted); and the cell pellet. The samples were layered onto CytoRich® Density Reagent according to the clinical trial protocol. Cytology preparations were prepared and cell counts made. Results show that 33 percent of epithelial cells are found in the supernatant, interface, and decant fractions and 67% in the final cell pellet. Abnormal cells are shown as 1% in supernatant fluid; 3% in the interface; 3% in the decant supernatant and 92% in the pellet.

The data show an average of one abnormal cervical cell for every 46 inflammatory cells in the starting sample. After CytoRich® enrichment, the ratio of abnormal cells to inflammatory cells was improved to 1 in 28. Increases in the proportions of abnormal cells to the total cell population (epithelial, inflammatory, and abnormal cells) from the initial sample to the final cell pellet ranged from 13.3% to 391.3% with a mean enrichment of 57%.

Clinical Trial

- Objective

A prospective, multi-center clinical study was conducted in which the objective was to assess AutoCyte PREP™ System performance as compared to the conventional Pap smear for the detection of cervical cancer, pre-cancerous lesions and atypical cells in a variety of patient populations. In addition, an assessment of specimen adequacy was performed.

- Method

The initial clinical study protocol was a masked, split sample, matched pair study, for which a conventional Pap smear was prepared first, and the remainder of the sample (the portion that would normally have been discarded) was collected into CytoRich® Preservative Fluid Collection Vials for transport to the laboratory.

At the laboratory, the preserved cell suspension was processed according to the AutoCyte PREP™ System protocol. The resulting AutoCyte PREP™ System slide and the matching conventional Pap smear slide were screened and diagnosed independently. A site pathologist evaluated all abnormal slides. Diagnostic results and findings reported were consistent with The Bethesda System categories.

Diagnostic "truth" for each case was determined by an independent reference pathologist as described by Shatzkin.[13] The independent reference pathologist at the designated referral site reviewed all abnormal and discrepant cases, repair cases and 5% of the normal cases from all sites in a masked fashion to form an independent diagnosis for each case providing an additional objective review of the results.

- Laboratory and Patient Characteristics

A total of 8,807 samples were evaluated across eight different study sites. The ages of women in the study ranged from 16 to 87 years, with 772 being post-menopausal. Of the 8,807 patients represented in the study, 1,059 presented a history of prior abnormal Pap smears. Five of the eight cytology laboratories included in the clinical study were either regional screening laboratories or screening hospitals and tested general patient populations. Three of the sites were clinical reference laboratories that tested high risk and referral patient populations (greater than 6% LSIL+). One of these is located in Canada. The other two clinical reference laboratories located in the Unites States evaluated cervical specimens from Vietnam and Kenya only. The entire patient population studied consisted of the following racial groups: Caucasian (44%), Black (30%), Asian (12%), Hispanic (10%), Native American (3%) and Other (1%).

Clinical Study Investigators and Enrollment

	Name and Address of Investigator	Subjects/Samples Enrolled
	Terrence J. Colgan, M.D. MDS Laboratories Belleville, Ontario, CANADA	1423
	Dugald A. Taylor, M.D. Laboratory Corporation of America Holdings Wichita, Kansas	571
	Sandra H. Bigner, M.D. Laboratory Corporation of America Holdings Burlington, North Carolina	1576
	Masood H. Sadeghi, M.D. Kaiser Permanente Regional Laboratory North Hollywood, California	497
	Karen M. McIntosh, M.D. Kaiser Permanente Regional Laboratory Berkeley, California	1388
	Lydia P. Howell, M.D. University of California, Davis, Medical Center Sacramento, California	704
	John W. Bishop, M.D. Creighton Unibersity/Saint Joseph Hospital Omaha, Nebraska	1670
	Mujtaba Husain, M.D. HistoPathology Associates (HPA) Southfield, Michigan	978

David C. Wilbur, M.D., University of Rochester Medical Center in Rochester, New York, was the independent pathologist.

Exclusions were made for incorrect paperwork, patients under age 16, patients with hysterectomies, and cytologically unsatisfactory and inadequate specimens. An effort was made to include as many cases of cervical cancer as possible by accessing high risk, infrequently screened, and referral patients as well as patients from Vietnam and Nairobi.

Clinical Study Results

- First Split-Sample Study

The goal of the clinical trial was to assess AutoCyte PREP™ performance as compared to the conventional Pap smear using diagnostic classification according to The Bethesda System. The study protocol was biased in favor of the conventional Pap smear because a conventional Pap smear was always prepared first, thereby restricting the AutoCyte PREP™ System slide to residual material remaining on the broom-type device (the portion of the sample that normally would have been discarded).[12] The intended use of the AutoCyte PREP™ System is a direct-to-vial application where all collected cells will be available to the AutoCyte PREP™ slide.

To compare the sensitivities of the AutoCyte PREP™ System and conventional Pap smear slides when read manually, the level of abnormality for the cases was determined by the reference pathologist and compared to diagnoses made by the study sites. The reference diagnosis was based upon the most abnormal diagnosis of either slide preparation by the independent reference pathologist. This result was used as the "truth" diagnosis or reference value for the comparison of the site results using AutoCyte PREP™ System verses the conventional Pap smear. The null hypothesis that the sensitivities of the two methods of slide preparation are equivalent was tested using McNemar's chi-square test for paired data.[13] In this statistical test, discrepant results for the two preparation methods were compared.

Table 1 presents a direct comparison of all site results for AutoCyte PREP™ slides vs. conventional Pap smear slides for the diagnostic treatment categories Within Normal Limits (WNL), Atypical Squamous Cells of Undetermined Significance / Atypical Glandular Cells of Undetermined Significance (ASCUS / AGUS), Low-grade Squamous Intraepithelial Lesion (LSIL), High-grade Squamous Intraepithelial Lesion (HSIL), and Cancer (CA).

Table 1 **First Split-Sample Study: 8,807 Matched Samples – Site Results Comparison – No Reference Pathologist**

SITE NO.	RESULTS BY SITE							
	SLIDE TYPE	WNL	ASCUS	AGUS	LSIL	HSIL	CA	Total
1	PREP	873	56	2	42	5	0	978
	CONV	881	46	2	29	20	0	978
2	PREP	1514	47	4	81	24	0	1670
	CONV	1560	33	6	40	31	0	1670
3	PREP	668	15	1	13	7	0	704
	CONV	673	11	0	13	6	1	704
4	PREP	1302	60	2	19	5	0	1388
	CONV	1326	37	2	19	4	0	1388
5	PREP	465	25	1	5	1	0	497
	CONV	444	45	1	4	3	0	497
6	PREP	1272	179	6	83	35	1	1576
	CONV	1258	209	9	68	30	2	1576
7	PREP	438	66	17	13	14	23	571
	CONV	417	93	19	4	22	16	571
8	PREP	1227	61	3	86	44	2	1423
	CONV	1209	57	0	94	61	2	1423
Total	PREP	7759	509	36	342	135	26	8807
	CONV	7768	531	39	271	177	21	8807

Table 2 presents a direct comparison of all site results for AutoCyte PREP™ System slides vs. conventional Pap smear slides for all diagnostic treatment categories.

21

Table 2 **First Split-Sample Study: 8,807 Matched Samples – All Site Results Comparison – No Reference Pathologist**

AUTOCYTE PREP	CONVENTIONAL						
	WNL	ASCUS	AGUS	LSIL	HSIL	CA	Total
WNL	7290	361	20	63	24	1	7759
ASCUS	343	101	4	44	15	2	509
AGUS	26	6	4	0	0	0	36
LSIL	87	52	2	147	53	1	342
HSIL	20	10	7	17	79	2	135
CA	2	1	2	0	6	15	26
Total	7768	531	39	271	177	21	8807

No independent reference pathologist results are reflected in **Table 1** or **Table 2.**

Table 3 **First Split-Sample Study: Comparison of All Site Results for Cases Designated by the Reference Method as ASCUS/AGUS – Discordant Error Analysis**

AUTOCYTE PREP	CONVENTIONAL PAP SMEAR		
	SUCCESS (ASCUS/AGUS)	ERROR (WNL & Reactive/Reparative)	Total
SUCCESS (ASCUS/AGUS)	113	205	318
ERROR (WNL & Reactive/Reparative)	180	229	409
Total	293	434	727

Result of McNemar test: $X^2mc = 1.62$, $p = 0.2026$
Errors Conventional: 205
Errors PREP: 180

Table 3 shows the results for cases identified by the reference pathologist to be ASCUS or AGUS. This evaluation allows analysis of the discordant errors to assess the sensitivity of the methods in the split-sample study design. Errors include WNL and Reactive / Reparative. Since the p-value determined by the McNemar test exceeded 0.05, the AutoCyte PREP™ System and conventional Pap smear results were considered equivalent.

Table 4 **First Split-Sample Study: Comparison of All Site Results for Cases Designated by the Reference Method as LSIL – Discordant Error Analysis**

AUTOCYTE PREP	CONVENTIONAL PAP SMEAR			
		SUCCESS (LSIL)	ERROR (WNL, Reactive/ Reparative & ASCUS/AGUS)	Total
	SUCCESS (LSIL)	140	63	203
	ERROR (WNL, Reactive/ Reparative & ASCUS/AGUS)	54	86	140
	Total	194	149	343

Result of McNemar test: $X^2mc = 0.69$, $p = 0.4054$
Errors Conventional: 63
Errors PREP: 54

Table 4 shows the results for cases identified by the reference pathologist to be LSIL. Errors include WNL, Reactive / Reparative and ASCUS / AGUS. As with ASCUS/AGUS, the sensitivity of the two methods in the split-sample study was statistically equivalent with a p-value in excess of 0.05.

Table 5 **First Split-Sample Study: Comparison of All Site Results for Cases Designated by the Reference Method as HSIL+ Discordant Error Analysis (LSIL is not an error)**

AUTOCYTE PREP	CONVENTIONAL PAP SMEAR			
		SUCCESS (HSIL+)	ERROR (WNL, Reactive/ Reparative & ASCUS/ AGUS)	Total
	SUCCESS (HSIL+)	160	28	188
	ERROR (WNL, Reactive/ Reparative & ASCUS/ AGUS)	36	38	74
	Total	196	66	262

Result of McNemar test: $X^2mc = 1.00$, $p = 0.3173$
Errors Conventional: 28
Errors PREP: 36

Table 5 shows results for cases identified by the reference pathologist to be HSIL+. In this comparison, LSIL was not considered an error but rather a discrepancy.[10,14,15] Error

includes WNL, Reactive / Reparative and ASCUS/AGUS. The sensitivity analysis of the discordant errors showed statistical equivalence of the methods in the split-sample study.

Table 6 First Split-Sample Study: Discordant Error Analysis for Cancer Cases (HSIL is not an error; LSIL is considered an error)

AUTOCYTE PREP	CONVENTIONAL PAP SMEAR		
	SUCCESS (CA)	ERROR (WNL, Reactive/ Reparative, ASCUS/AGUS & LSIL)	Total
SUCCESS (CA)	19	2	21
ERROR (WNL, Reactive/ Reparative, ASCUS/AGUS & LSIL)	5	1	6
Total	24	3	27

Result of McNemar's Test: $X^2mc = 1.645$, $p = 0.1980$
Errors Conventional: 2
Errors PREP: 5

Table 6 shows results (all sites) for cases judged to be cancer by the reference method. Errors include WNL, Reactive / Reparative, ASCUS/AGUS and LSIL. The sensitivity analysis of the discordant errors showed statistical equivalence of the methods. (These 27 cancer cases were included in the re-evaluation study. The data can be found in **Table 9.**)

Table 7 First Split-Sample Study: Comparison of All Site Results for Cases Designated by the Reference Method as HSIL+ Discordant Error Analysis (LSIL was considered an error in this analysis)

AUTOCYTE PREP	CONVENTIONAL PAP SMEAR			
		SUCCESS (HSIL+)	ERROR (WNL, Reactive/ Reparative, ASCUS/ AGUS & LSIL)	Total
	SUCCESS (HSIL+)	94	33	127
	ERROR (WNL, Reactive/ Reparative, ASCUS/ AGUS & LSIL)	67	68	135
	Total	161	101	262

Result of McNemar test: $X^2mc = 11.56$, $p = 0.0007$
Errors Conventional: 33
Errors PREP: 67

Table 7 shows results for cases identified by the reference pathologist to be HSIL+. Error includes WNL, Reactive / Reparative, ASCUS/AGUS and LSIL. Though not stated in the original study protocol,[10] a statistical comparison of methods was performed where LSIL was considered a diagnostic error against a case determined to be HSIL+ by the single independent reference pathologist. In this statistical comparison of diagnostic sensitivities, if LSIL was considered an error, as opposed to a minor discrepancy, AutoCyte PREP™ System would not be considered equivalent to the conventional Pap smear for detection of HSIL abnormality in the split-sample study.

- Masked Re-evaluation of HSIL+ Cases

A new evaluation was conducted to determine if the results were affected by preparation quality or interpretational subjectivity. In order to assess the 262 cases which were diagnosed as HSIL+ in the original study (**Table 7**), an additional evaluation was conducted after implementing a new training program for cytology professionals designed to emphasize consistent interpretation between the diagnostic groups of The Bethesda System. These HSIL+ cases were re-masked as part of a re-evaluation consisting of a total of 2,438 specimens prepared using the same split sample protocol. Conventional and AutoCyte PREP™ System study site results were then compared to a new reference value which required agreement of at least two of three independent reference pathologists as to the most abnormal cytology diagnosis.

In the reference process for the re-evaluation, both slide preparations from the discordant cases (AutoCyte PREP™ System and the conventional Pap smears) were re-screened by a second cytotechnologist, and newly identified abnormalities were added to those from the initial screening. Three reference cytopathologists then evaluated all discordant cases using a masked protocol. This more stringent reference method reduced the number of HSIL+ reference cases from 262 in the original study to 209 in the re-evaluation. The 53 case difference may be explained as follows: 48 cases were diagnosed by the more stringent reference method as LSIL or less severe; the adequacy of 3 cases was judged unsatisfactory upon re-evaluation; and the remaining 2 cases were not available for assessment in the masked re-evaluation study. The laboratories and investigators are listed below.

Clinical Study Investigators and Enrollment

	Name and Address of Investigator	Subjects/Samples Enrolled
1.	Jan Hessling, M.D. Laboratory Corporation of America, Holdings Research Triangle Park, North Carolina	974
2.	Dominic Raso, M.D. Pathology Consultants of Central Virginia Lynchburg, Virginia	247
3.	Brent Schiffer, M.D. International Cancer Screening Laboratories San Antonio, Texas	1187

The reference pathologists for the re-evaluation study were: Dugald Taylor, M.D., Laboratory Corporation of America, Holdings/Litton Pathology of North Wichita, Kansas; Joseph Callicott, M.D., Pathology Consultants Of Central Virginia, Lynchburg, Virginia; Mujtaba Husain, M.D., HistoPathology Associates, Southfield, Michigan.

Table 8 **Re-Evaluation Study: Discordant Error Analysis for 209 Original HSIL+ Cases Re-Evaluated by the More Stringent Reference Criteria Involving Three Independent Reference Pathologists**

AUTOCYTE PREP	CONVENTIONAL PAP SMEAR			
		SUCCESS (HSIL+)	**ERROR (WNL, Reactive/ Reparative, ASCUS/ AGUS & LSIL)**	**Total**
	SUCCESS (HSIL+)	153	26	179
	ERROR (WNL, Reactive/ Reparative, ASCUS/ AGUS & LSIL)	24	6	30
	Total	177	32	209

Result of McNemar Test: $X^2mc = 0.02$, $p = 0.8875$

Errors Conventional: 26

Errors PREP: 24

Table 8 shows results for cases identified by the reference pathologist to be HSIL+. Error includes WNL, Reactive / Reparative, ASCUS/AGUS and LSIL. In this comparison, LSIL was considered a diagnostic error against a case determined to be HSIL+ by the independent reference pathologist. Comparison of diagnostic sensitivities showed statistical equivalence between the two methods.

Table 9 Re-Evaluation Study: Discordant Error Analysis for Cancer Cases (HSIL is not an error; LSIL is considered an error)

AUTOCYTE PREP	CONVENTIONAL PAP SMEAR		
	SUCCESS (CA)	ERROR (WNL, Reactive/ Reparative, ASCUS/AGUS & LSIL)	Total
SUCCESS (CA)	32	3	35
ERROR (WNL, Reactive/ Reparative, ASCUS/AGUS & LSIL)	3	0	3
Total	35	3	38

Result of McNemar's Test: $X^2mc = 0.00$, $p = 1.0000$
Errors Conventional: 3
Errors PREP: 3

Table 9 shows results for cases judged to be cancer by the new reference method (all sites). Errors include WNL, Reactive / Reparative, ASCUS/AGUS and LSIL. One error resulted from a LSIL interpretation. All other errors involved interpretation of slides as ASCUS / AGUS or WNL.

The masked re-evaluation contained 2097 new cases that were used to re-mask the original HSIL+ samples. The analysis and comparison of the preparations from these new cases follows in **Table 10.**

Table 10 Re-Evaluation Study: 2097 Direct Site Results Comparison – No Reference

AUTOCYTE PREP	CONVENTIONAL						
	WNL	ASCUS	AGUS	LSIL	HSIL	CA	Total
WNL	1561	128	0	47	30	0	1766
ASCUS	80	37	1	6	8	1	133
AGUS	9	7	0	0	1	0	17
LSIL	33	11	1	33	11	1	90
HSIL	26	18	1	18	19	3	85
CA	1	2	0	0	1	2	6
Total	1710	203	3	104	70	7	2097

Of the 2097 new cases described above, 77 were diagnosed HSIL+ by the reference pathologists. **Table 11**presents the sensitivity analysis for those 77 HSIL+ cases.

Table 11 Re-Evaluation Study: Comparison of All Site Results for Cases Designated by the Reference Method as HSIL+ Discordant Error Analysis (LSIL was considered an error in this analysis)

AUTOCYTE PREP	CONVENTIONAL PAP SMEAR		
	SUCCESS (HSIL+)	ERROR (WNL, Reactive/ Reparative, ASCUS/ AGUS & LSIL)	Total
SUCCESS (HSIL+)	25	21	46
ERROR (WNL, Reactive/ Reparative, ASCUS/ AGUS & LSIL)	21	10	31
Total	46	31	77

Result of McNemar test: $X^2mc = 0.00$, $p = 1.0000$
Errors Conventional: 21
Errors PREP: 21

Analysis of the discordant errors in **Table 11** showed an equal number of HSIL+ misses for both the AutoCyte PREP™ slide and conventional Pap smear. Error includes WNL, Reactive / Reparative, ASCUS/AGUS and LSIL. The statistical test demonstrated equivalence between the two methods in the split-sample design even when LSIL is considered an error against a reference value of HSIL+.

Table 12 summarizes the descriptive diagnoses of benign findings for all sites.

Table 12 First Split-Sample Study: Summary of Benign Cellular Changes

Descriptive Diagnosis	**AutoCyte PREP™**		**Conventional**	
Number of Patients: 8,807	N	%	N	%
Benign Cellular Changes				
*Infection:				
Candida species	440	5.0	445	5.1
Trichomonas vaginalis	118	1.3	202	2.3
Herpes	8	0.1	6	0.1
Gardnerella	85	1.0	44	0.5
Actinomyces species	6	0.1	2	<0.1
Bacteria (other)	52	0.6	191	2.2
**Reactive Reparative Changes	424	4.8	319	3.6

*For Infection category above, observations of infectious agents are reported. More than one class of organism may be represented per case.

**Reactive reparative changes included reactive changes associated with inflammation, atrophic vaginitis, radiation and IUD use, as well as typical repair involving squamous, squamous metaplastic or columnar epithelial cells.

A total of 8,807 cases contained no "unsatisfactory" assessment by either the trial sites or the reference site. An additional 239 samples were scored "unsatisfactory" by either or both the trial sites or reference site on either or both preparations. Of those 239 unsatisfactory cases, 151 were noted on conventional slides only; 70 on AutoCyte PREP™ slides only; and 18 were observed on both the conventional and AutoCyte PREP™ slides. All unsatisfactory cases were excluded from diagnostic comparison by The Bethesda System categories, but were added back for comparison of preparation adequacy.

Tables 13 through **16** show preparation adequacy results for all sites.

Table 13 First Split-Sample Study: Preparation Adequacy Results

Preparation Adequacy Number of Patients: 9,046	AutoCyte PREP™		Conventional	
	N	%	N	%
Satisfactory	7607	84.1	6468	71.5
Satisfactory for Evaluation But Limited By:	1385	15.3	2489	27.5
Endocervical Component Absent	1283	14.2	1118	12.4
Air-Drying Artifact	0	0	17	0.2
Thick Smear	1	<0.1	0	0
Obscuring Blood	53	0.6	121	1.3
Obscuring Inflammation	102	1.1	310	3.4
Scant Squamous Epithelial Cells	4	<0.1	7	0.1
Cytolysis	10	0.1	11	0.1
No Clinical History	0	0	0	0
Not Specified	60	0.7	1018	11.3
Unsatisfactory for Evaluation:	54	0.6	89	1.0
Endocervical Component Absent	42	0.5	42	0.5
Air-Drying Artifact	0	0	0	0
Thick Smear	0	0	2	<0.1
Obscuring Blood	7	0.1	6	0.1
Obscuring Inflammation	6	0.1	6	0.1
Scant Squamous Epithelial Cells	6	0.1	0	0
Cytolysis	0	0	1	<0.1
No Clinical History	0	0	0	0
Not Specified	37	0.4	32	0.5

Note: Some patients had more than one subcategory.

The additional unsatisfactory cases determined by the reference pathologist, and the total number of unsatisfactory results are reflected in **Table 15**.

Table 14 First Split-Sample Study: Summary of Preparation Adequacy Results – All Clinical Trial Sites

AutoCyte PREP	CONVENTIONAL			
	SAT	SBLB	UNSAT	Total
SAT	5868	1693	46	7607
SBLB	579	772	34	1385
UNSAT	21	24	9	54
Total	6468	2489	89	9046

SAT = Satisfactory, SBLB = Satisfactory But limited By(some specified condition), UNSAT = Unsatisfactory

UNSAT: Result of McNemar Test X^2 mc = 8.57, p = 0.0034
SBLB: Result of McNemar Test X^2 mc = 546.21, p = 0.0000

Table 14 shows results from a comparison of preparation adequacy for the conventional Pap smear and AutoCyte PREP™ slides. There were significantly fewer Unsatisfactory and SBLB cases with AutoCyte PREP™ System as compared to the conventional Pap smear.

Table 15 First Split-Sample Study: Comparison of Unsatisfactory Results From The Clinical Trial Sites and The Reference Site

AutoCyte PREP™	CONVENTIONAL		
	SAT	UNSAT	Total
SAT	8807	151	8958
UNSAT	70	18	88
Total	8877	169	9046

Result of McNemar Test X^2 mc = 29.69, p = 0.0000

Table 15 shows comparison of satisfactory and unsatisfactory preparations from the evaluations at both the trial sites and the reference site. AutoCyte PREP™ slides show a statistically significant reduction of unsatisfactory cases compared to the conventional Pap smear.

Table 16 Preparation Adequacy Results by Site - SBLB Rates for No Endocervical Component (ECC)

Site	Cases	AutoCyte PREP™ SBLB no ECCs N (%)	Conventional SBLB no ECCs N (%)
1	995	60 (6.0)	85 (8.5)
2	1712	121 (7.1)	54 (3.2)
3	712	180 (25.3)	141 (19.8)
4	1395	165 (11.8)	331 (23.7)
5	500	58 (11.6)	56 (11.2)
6	1695	473 (28.2)	238 (14.2)
7	589	19 (3.3)	3 (0.5)
8	1448	207 (14.3)	210 (14.5)
All Sites	9046	1283 (14.2)	1118 (12.4)

Detection of endocervical cells **(Table 16)** varied at different trial sites. Overall, there was a 1.8% difference in endocervical cell detection between the conventional Pap smear and AutoCyte PREP™ methods, which is similar to previous studies involving split-sample methodology.[16,17]

IX. CONCLUSIONS DRAWN FROM STUDIES

Validity of Clinical Data

The AutoCyte PREP™ System provides similar results to the conventional Pap smear in split-sample comparisons in a variety of patient populations and laboratory settings. In addition, there were significantly fewer Unsatisfactory and SBLB cases with AutoCyte PREP™ System as compared to the conventional Pap smear. The AutoCyte PREP™ System may thus be used as a replacement for the conventional Pap smear method of slide preparation for the detection of atypical cells, precancerous lesions, cervical cancer, and all other cytologic categories defined by The Bethesda System.

Safety and Effectiveness

The goal of the clinical trial was to demonstrate safety and effectiveness in a comparison of the AutoCyte PREP™ System with the conventional Pap smear. To compare the sensitivities of the AutoCyte PREP™ System and conventional Pap smear slides when read manually, the level of abnormality was determined by the reference laboratory diagnosis and compared to diagnoses made by the study site laboratory. The null hypothesis that the sensitivities of the two methods of slide preparation are equivalent was tested using McNemar's chi-square test for paired data.[12] In this statistical test, discrepant results for the two preparation methods were compared. These discrepant results occur when one of the slides agrees with the reference laboratory's minimum level of abnormality and the other slide interpretation does not meet the threshold of abnormality, thereby suggesting an error related to the preparation.

Risk / Benefit Analysis

The results of the clinical investigation demonstrated that cervical cytology slides prepared with the AutoCyte PREP™ System provide similar results to the conventional Pap smear for the detection of cervical neoplasia and its precursor lesions. The conventional Pap smear and another approved thin-layer liquid preparation device are two alternative procedures for preparing cervical cytology specimens for screening.

X. Panel Recommendation

Pursuant to section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology and Pathology Devices Panel, an FDA advisory panel, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XI. CDRH ACTION ON THE APPLICATION

CDRH issued an approval order for the applicant's PMA on June 17, 1999.

The applicant's manufacturing and control facilities were inspected and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs).

XII. APPROVAL SPECIFICATIONS

Directions for use: See attached labeling.

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order.

XIII. BIBLIOGRAPHY

1. Kurman RJ, Solomon D: The Bethesda System for reporting cervical/vaginal cytologic diagnoses. Springer-Verlag, 1994.
2. Papanicolaou GN: A New Procedure for Staining Vaginal Smears. Science 1942; 95:438-439.
3. Cramer DW: The role of Cervical Cytology in the Declining Morbidity and Mortality of Cervix Cancer. Cancer 1974; 34:2018-2027.
4. Dickinson LE: Control of Cancer of the Uterine Cervix by Cytologic Screening. Gynecol Oncol 1975; 3:1-9.
5. Grohs HK, Zahniser DJ, Geyer JW: Standardization of Specimen Preparation Through Mono/Thin-layer Technology in Automated Cervical Cancer Screening. Edited by HK Grohs, OAN Husain. New York, Igaku-shoin, 1994, pp. 176-185.
6. McGoogan E, Reith A: Would Monolayers Provide More Representative Samples and Improved Preparations for Cervical Screening? Overview and Evaluation of Systems Available. Acta Cytol 1996; 40:107-119.
7. Barr WT, Powell DEB, Raffan JB: Cellular Contamination During Automatic and Manual Staining of Cytological Smears. J Clin Pathol 1970; 23:604-607.
8. Husain OAN, Grainger JM, Sims J: Cross Contamination of Cytological Smears, with Automated Staining Machines and Bulk Manual Staining Procedures. J Clin Path 1978; 31:63-68.
9. Hutchinson ML, Agarwal P, Denault T, Berger B, Cibes ES: A New Look at Cervical Cytology: ThinPrep Multicenter Trial Results. Acta Cytol 1992; 36:499-504.
10. Hutchinson ML, Isenstein LM, Goodman A, Hurley AA, Douglass KL, Mui KK, Patten FW, Zahniser DJ: Homogeneous Sampling Accounts for the Increased Diagnostic Accuracy Using the ThinPrep™ Processor. Am J Clin Pathol 1994;101:215-219.
11. Sprenger E, Schwarzmann P, Kirkpatrick M, Fox W, Heinzerling RH, Geyer JW, Knesel EA: The False Negative Rate in Cervical Cytology: Comparison of Monolayers to Conventional Smears. Acta Cytol 1996; 40:81-89.
12. Naujoks H, Strohmeier R, Bicker T, van Driel-Kulker AMJ, Knepfle CFHM, Ploem JS: Interobserver Variability in the Cytological Diagnosis of 1500 Papanicolaou Stained Cervical Monolayer Specimens. Pathol Res Pract 1990; 186:150-153.
13. Shatzkin A, Conner RJ, Taylor PR, Bunnag B: Comparing new and old screening tests when a reference procedure cannot be performed on all screeners. Am J Epidemiol 1987; 125: 672-678.

14. McNemar Q: Note on the sampling error of the difference between correlated proportions or percentages. Psychometrika 1947; 12:153-7.

15. Mayeaux EJ, Harper MB, Fleurette A, Pope JB, Phillips GS: A comparison of the reliability of repeat cervical smears and colposcopy in patients with abnormal cervical cytology. J Fam Pract 1995; 40: 57-62.

16. College of American Pathologists. Interlaboratory comparison program in cervicovaginal cytology (PAP). 1995.

17. Austin RM, Ramzy I: Increased detection of epithelial cell abnormalities by liquid-based gynecologic cytology preparations. A review of accumulated data. Acta Cytol 1998; 42: 178-184.